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Rhizosphere Microbial Genetic Resources as PGPR Potential Isolated from Maize Inbred Populations Var.Bisma

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Abstract

A number of rhizosphere bacteria affect plant growth through contributing to the host plant-source of endogenous phytohormones. This study aims to obtain potential microbial genetic resources which isolated from corn. A total of 24 potential microbial genetic resource collections were isolated from the rhizosphere population of inbred corn plant var. Bisma obtained from the Biogen-Plant GeneBank collection. The potential of microbial genetic resources observed include the ability to produce IAA, N_2 fixation, Phosphate solubility, and their ability to stimulate corn seed germination. Rhizosphere bacteria were isolated using the soil extract media. Isolates that showed the ability to produce IAA, N_2 fixation and high phosphate solubility were tested for corn seed germination. The results showed that there were 24 isolates obtained from corn rhizosphere which dominated by bacilliform-shaped gram-positive bacteria capable of IAA producing ranged from 4.83 to 125.84 ppm. Almost 16 rhizosphere bacterial isolates were capable of dissolving phosphate with the phosphate solubility index ranged from 2.1 to 4.6. The selected potential isolates of J11, J16 and J19 were able to stimulate the corn seed germination.

Keywords: microbial genetic resources; rhizosphere; maize; IAA; plant growth

1. Introduction

Commodity crops have a primary role to provide food, feed as well as industrial needed in the country that tend to increase annually along with population growth and growing of food and feed industry in terms of national food security. Maize (*Zea mays*) is one of the important agricultural crops in Indonesia second most important food grains crops after rice. Corn plant occupies an important position in the national economy as a source of carbohydrate [1]. In some areas in Indonesia corn used as staple food, as well as animal feed and industrial purposes [2].

Indonesia has the opportunity as suppliers of world corn demand because of the available land suitability for planting corn. In the cultivation of maize, fertilizer become as one of the most important means for crop production, so that their manure becomes necessary for crop sustainability and soil productivity. To date, the fertilizer distribution becomes shortages in some areas, and the fertilizer price is becoming increase expensively. This condition is an opportunity to supply production of various types of biological fertilizers to complement the shortage of fertilizers [3, 4].

In effort to handling biological fertilizer scarcity that occurred in the agricultural community, there is an opportunity to use the rhizosphere microbial inoculants to enhance plant growth [5, 6, 7]. Microbial rhizosphere may play an important role to provide mineral elements, produce vitamins and growth hormones, and inhibit the

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growth of pathogens [8]. Munif and Awaludin 2011 [9], states that the rhizosphere microbes plays a role in providing plant nutrients e.g. N, P and K that available for plants, increase the plants ability to utilize the nutrients, and increase the resilience of crops to drought and disease. Rhizosphere bacteria can stimulate plant growth by producing Indole acetic acid (IAA) as a secondary metabolite, and the availability of N and P to plants. IAA is one a plant hormone that is indispensable for plant growth. According to Siregar 2009 [10], the IAA can regulate many physiological processes, such as cell growth, division and differentiation as well as protein synthesis. Nitrogen and phosphate is an essential nutrient for plant growth.

Utilization of bacteria that potentially showed ability to fix up nitrogen and phosphate availability, and produce IAA growth hormone will facilitate in providing a biological fertilizer for plants [11, 12]. Peix et al., 2001 [13] stated that the application of biological fertilizer will gain double in providing an element of N and P as well as generate IAA. The important role of Plant Growth Promoting Rhizobacteria (PGPR) may increase plant's ability to use water, nutrient availability and plant resistance to pathogen attack. This study aims to obtain rhizosphere bacterial isolates of corn, characterize the morphology and biochemical properties of their ability to produce IAA, provides nutrients such as N and P, to obtain bacteria inoculants to booster corn growth and to get information regarding the molecular identification of selected bacterial inoculants.

2. Methods

2.1. Soil sampling and isolation of rhizosphere bacteria

Soil samples were taken from the inbred corn plant rhizospheres generated from open pollinated Inbred S6 maize var. Bisma. Corn rhizosphere samples were put into a sterile plastic bag to be brought into the laboratory for isolation and characterization of microbes.

Rhizosphere bacterial isolation was done by taking part of the plant rhizosphere. A total of 10 g soil rhizosphere section was diluted into a 90 mL of sterile distilled water and it was further agitated at a speed of 150 rpm with shaking for 15 minutes. A total of 1 mL of the extract was put into a test tube containing 9 mL of sterile distilled water, then homogenized with a vortex. The 1 mL tube was transferred to the serial dilution of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} , 10^{-5}, then 10 the bacterial suspension was planted in the soil extract agar media (SEA, HIMEDIA) consisting of 1 g of glucose, 0.5 g dipotassium phosphate, 17.75 g soil extract and 15 g agar). Then aliquot of 10µL was spread out in media using a dry sterile glass. Incubation was performed at 30°C for 1-3 days. Bacterial colony that showed different visual appearance was purified back to the NA media for bacterial purification. The pure bacterial colony then stored in slant agar medium.

2.2. Number of rhizosphere bacterial populations and characterization of bacteria

Bacterial colonies were counted on petri dishes for approximately 30-300 colonies. Colonies that grew on SEA media was counted for bacterial population as units of CFU (colony forming units). The estimates of the bacterial number of colonies were calculated based on a Fardiaz 1992 formula [14], namely:

$$\text{Estimation of the number of cells} = \frac{\text{Number of colonies x 1}}{\text{dilution factor (CFU/mL)}}$$

Characterization of bacteria were based on morphological colonies differences

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grown on SEA media. The morphology of colonies grown in a medium that is observed in the form of: a. Size: pinpoint/punctiform (point), small (small), moderate (moderate), large (huge); b. Form: f colonies that appear in the form of a circular, filament; c. Elevation: elevation view in form of a flat, raised; d. Margins: in form of waves, slippery, irregular etc [15].

2.3. Biochemical testing

The biochemical testing was characterized by phosphate solubilization assay. The ability to dissolve inorganic phosphate (tricalcium phosphate) was assessed using the Pikovskaya media. One bacterial ose from the slant agar culture was inoculated into 300 µL NB media. A 3 µL the mixture was spreaded on Pikovskaya medium, incubated at 28°C for 7 days [16]. Bacterial colony with a clear zone was scored as positive reaction for dissolving phosphate, and the diameter of clear zone was measured. Phosphate Solubility index (PSI) was calculated using the formula Vazquez et al., 2000[17] i.e;

$$\text{PSI} = \frac{\text{Colony diameter clear zone}}{\text{colony diameter}}$$

2.4. Nitrogen fixation assay

Selection of N fixing-bacteria was carried out using semi-solid free bromthymol nitrogen-blue (NFB) media. One loopful bacterial ose from the agar slant culture was inoculated into 10 mL of NB and agitated at 500 rpm for 24 hours at 28°C. A total of 1mL of the extract was added to the test tube 9 mL of sterile distilled water and then homogenized with a vortex, until serial dilution of 10^{-3}, 10^{-4} and 10^{-3}, 10^{-5} of the bacterial dilutions. A 100 µL suspension of 10^{-4} and 10^{-5} was spreaded on NFB media in triplo, incubated for 7 days at 28°C [16]. Isolates showing blue color changes in medium with their white pellicle near the surface were considered as positive reaction. N was calculated from the microbial population using the Most Probable Number method.

2.5. IAA producing assay

The IAA standard was prepared by weighing as much as 0.01 g synthetic IAA diluted into 100 mL of distilled water. Leaching was done slowly with ethanol. A 100 ppm IAA solution then dissolved into a concentration of 0.2 ppm, 1 ppm, 5 ppm, 15 ppm, 25 ppm, 35 ppm and 45 ppm. Sample test was done by incubating 24 h-old culture isolates in 10mL of NB medium and agitated at 500 rpm. The bacterial suspension was incubated for 24 h and 1000µL was inoculated on minimal media. The bacterial suspension which had been inoculated in minimal media was agitated at 500 rpm for 24 h. A total 2000µL bacterial culture on minimal media put in eppendorf tubes in duplicate. Each sample was centrifuged for 10 min at a speed of 10.000 rpm in a temperature 4^{o}C. As many as 2 mL of supernatant was taken and put into a test tube and then added with 4mL Salkowski reagent. The homogenized sample in a test tube was vortexed and incubated for 60 min in temperatures of 25ºC, and then samples were incubated and subsequently measured for its absorbance using spectrophotometer at a wavelength of 530 nm [18].

2.6. Screening of rhizosphere bacteria as inoculants boosters for plants growth in vitro

Corn seeds surface was sterilized using 70% absolute ethanol for 5 minutes, then with 2% NaOCl for 3 min and washed 3 times with sterile water. Corn seeds were soaked in the selected rhizosphere bacterial culture and control of water for 2 h, respectively. Corn seed was placed on the surface of a sterile filter paper in a petri dish (4 corn kernels / Petri dish) incubated for 5 days. The selected rhizosphere bacterial culture was made by rejuvenating the bacterial isolates on NA media. The 24 h-old

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bacterial isolates was added with 10mL sterile distilled water and homogenized with a vortex. The 1000 µL bacterial culture was taken, and check for its cell density using the standard Mc Farland until 10^8 CFU/mL [19]. Observation was carried out to the speed of germination compared with control without inoculation microbes (water). Observation was done by measuring the length of root and stem, measured at 5th day. Bacterial culture that give a positive response in the form of roots and stems growth were tested again in the greenhouse.

2.7. Screening of selected rhizosphere bacterial isolates as inoculants boosters for plants growthin the green house

Three (3) maize seeds were planted in circular pots with a diameter of 30 cm containing sterile soil seed corn. Corn seeds that have germinated / 7 old-days were inoculated with selected bacterial culture. Bacterial culture was prepared and adjusted using the standard McFarland to approximately 10^8 CFU/mL. Variable growth observed was the form of plant height and root length and dry weight of corn which was measured at 4 week plant, compared with the treatment without microbial inoculation (water). Crop-harvest was done by removing the corn from the pot by means of loosening soil in the pot and then corn roots was soaked with water until the soil attached to the roots lost. The crop was put in a paper bag and packed to be stored in a 70°C oven for 2 days and measured for its dry weight. The design of experiments was conducted using Complete Random Design (CRD), and the data obtained were analyzed using ANOVA test with a 95% level of confidence. The significant difference then was analyzed using Honestly Significant Difference (HSD) test.

2.8. Identification of the selected bacterial isolates

Three (3) selected isolates were morphological differentiated using gram reaction, then they were tested for DNA isolation using DNA isolation KIT (Promega, USA) and further sequenced and analyzed by BLAST program.

3. Result and Discussion

3.1. Cornrhizosphere bacterial isolation

Corn rhizosphere bacterial isolation which performed on soil extract agar medium provides all the essential nutrients required for the growth of soil microbes. The highest of rhizosphere bacterial population was observed from > 120 days age of corn crop (code 64). The corn code 64 indicated the highest rhizosphere bacterial populations, with a population of bacteria about 7.8 x 10^5CFU/mL, whereas rhizosphere bacterial populations maize code 168was the lowest, with a bacteria population of 1.36 x 10^5 CFU/mL (Table 1).

Table 1. Corn rhizospere bacterial population

Corn code	Plant Age (days)	Number of colony (CFU/mL) x 10^5
28	>120	4,3
64	>120	7,8
67	>120	2,3
125	90 - 120	1,6
128	90 - 120	2,1
135	90 - 120	4,1
136	> 120	7,6
168	75 - 90	1,3
186	75 - 90	1,5

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According to Purwaningsih 2005 [20], the higher the number of the bacterial population is a sign of the higher level of fertility of the soil, because microbes can degrade organic compounds into nutrients which available to the plants, and provide enough organic matter and other compounds for microbial growth in the soil. Rhizosphere bacterial populations of maize code 64 showed the highest population. According to Schroder and Hartmann 2003 [21], the different population of the rhizosphere bacteria on maize may influenced by the interaction between plants and microorganisms that stimulated root exudates. The root exudates will affect the growth and activity of microorganisms in the surrounding rhizospherearea. Table 1 show that the crop age > 120 days has a population of bacteria higher than that of corn plant age 90-120 days, and age of 75-90 days. In accordance with the statement of Widyati 2013 [22], the plant variety also may determine the rhizosphere microbial community diversity. Bacterial colonies growing on soil extract agar medium were obtained as much as 24isolates. The isolates showed varying characteristics of both morphology and color properties. Their varying characteristics in isolates obtained showed that microorganisms which occupy the rhizosphere of corn may vary due to abundant nutrients in the soil. The bacterial colonies growing in a variety of media showed white, solid white, milky white, transparent white, creamy white and beige color. Margins colonies bacterial growth was uneven and irregular (undulate). Elevation of bacterial colonies growing is flat and embossed (raised). The growing bacterial colonies shape was irregular and circular (Table 2).

Table 2. Characteristics of corn-rhizosphere bacterial colony

Sample Code	Morphologicalcharacteristic				Gram	Cells shape
	Color	Margin	Elevation	Colony shape		
J1	transparent white	flat	flat	irregular	-	bacilliform
J2	white	flat	flat	circular	-	bacilliform
J3	creamy	flat	flat	circular	+	bacilliform
J4	white	flat	flat	circular	+	bacilliform
J5	creamy white	flat	raised	circular	+	bacilliform
J6	white	undulate	flat	irregular	+	bacilliform
J7	creamy white	flat	flat	circular	+	bacilliform
J8	creamy	flat	flat	circular	+	bacilliform
J9	white	undulate	flat	irregular	+	bacilliform
J10	creamy	undulate	flat	circular	-	bacilliform
J11	creamy white	flat	flat	circular	+	bacilliform
J12	creamy white	flat	flat	circular	+	bacilliform
J13	creamy white	flat	flat	circular	+	bacilliform
J14	creamy	flat	raised	circular	-	bacilliform
J15	creamy	undulate	flat	irregular	+	bacilliform
J16	milky white	undulate	flat	irregular	+	bacilliform
J17	milky white	undulate	flat	irregular	+	bacilliform
J18	milky white	flat	flat	circular	-	bacilliform
J19	milky white	flat	flat	circular	+	bacilliform
J20	white	flat	flat	circular	+	bacilliform
J21	creamy white	flat	flat	circular	+	bacilliform
J22	milky white	serrate	flat	irregular	-	bacilliform
J23	white	undulate	flat	irregular	+	bacilliform
J24	white	flat	flat	circular	+	bacilliform

The presence of rhizosphere bacterial diversity proved that the environment around roots are rich in minerals and other nutrients as a result of exudates released by the roots causing many microorganisms which occupies rhizosphere. In relation with the statement of Narula et al., 2009 [23] this root exudates contain compounds that are needed by microorganisms such as sugars, organic acids and amino acids, hence many microorganisms are attracted to live around the roots and interact with the roots.

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Cell morphology of pure isolates was observed by Gram stain [24]. Based on the results obtained with staining, the characteristics isolates was Gram-positive bacilli (18 isolates) and Gram negative bacilli form (6 isolates), respectively.

3.2. Screening cornrhizosphere bacteria

Twenty four (24) isolates obtained were tested for biochemical activity to produce IAA, nitrogen (N) fixing and dissolving phosphate (P) (Table 3).

Table 3.Consentration of IAA produced corn-rhizosphere bacteria

Isolates code	IAA concentration (ppm)
J1, J2, J5, J7, J10, J12, J14, J20, J22 and J23	1 – 25
J4, J6, J9, J13, J16, J17, J18 and J21	26 – 50
J19, J24 and J11	51 – 75
J15 and J3	76 – 100
J8	>100

Peix et al., 2001 [13] stated that the use of nitrogen-fixing bacteria, phosphates solubilization and potentially IAA growth hormone will make it easier to provide a biological fertilizer for plants; because it had dual advantages i.e.; provide an element of N and P as well as IAA producer. Biochemical activity by IAA assay was performed to determine the quantitatively IAA concentration excreted by bacteria into the growth medium. The ability to produce IAA qualitatively can be seen by comparing the pink color formed after addition of Salkowski reagent. Rahman et al., 2010 [25] stated that the color change occurs due to the reaction between the reagents Salkowski with IAA produced by isolates, $FeCl_3$ and H_2SO_4 which were building blocks of complex Salkowski reagent Tris (indole-3-aceto)-Fe (III). The reaction of the color change indicates that isolates could metabolize L-tryptophan to IAA.

The ability of each different isolates producing IAA can be seen in Table 3. One strain (J8) showed the ability to produce IAA > 100 ppm with the IAA concentration of 125.84 ppm. The lowest IAA concentration was shown by J14 isolate with the IAA of 4.83 ppm. The corn roots bacterial isolates produce IAA hormone at different level, presumably it was influenced by the physiological properties of each bacteria and the ability to convert tryptophan to IAA. The ability to produce IAA in high levels is possible enough to improve plantgrowth. This is consistent with the statement of Munif and Awaluddin, 2011 [9] that the IAA is hormone that is indispensable for growth. Besides being able to produce IAA, isolate also can dissolve phosphate. Phosphate soluble ability was measured by the clear zone formed on Pikovskaya media. The greater the clear zone, the greater its ability to dissolve phosphate. According to Zulaika 2015 [26], Pikovskaya medium is white turbid containing $Ca_3(PO4)_2$ bound. When phosphate apart in the medium, it will form a clear zone. Clear zone formed on media Pikovskaya was determined as Phosphate Solubility Index (PSI) [27].

Some isolates capable of forming a clear zone because they produce organic acids. The organic acid phosphate leads to dissolution of phosphate bound into an avalaible form [28]. The organic acid is able to bind into $CaCa_3(PO4)_2$ ions and liberating H_2PO_4 to form a colored clear area [29]. The ability of dissolving phosphate by the 24 corn - rhizosphere isolates showed that 16 bacterial isolates capable of dissolving phosphate (Table 4).

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Table 4. Index P solubility of maize crop by rhizosphere bacterial isolates

Isolates code	Clear Zones	Diameter		P index solubilization
		Clear zones (cm)	Bacterial colony (cm)	
J1	+++	1.8	0.7	4.6
J2	+++	1.7	0.7	3.4
J3	-	-	-	-
J4	++	1.0	0.5	3.0
J5	++	1.0	0.9	2.1
J6	-	-	-	-
J7	++	1.0	0.5	3.0
J8	+++	1.3	0.6	3.2
J9	+++	1.4	0.5	3.8
J10	+++	1.8	0.7	3.6
J11	+++	1.2	0.4	4.0
J12	-	-	-	-
J13	-	-	-	-
J14	+++	1.2	0.5	3.4
J15	-	-	-	-
J16	+++	1.4	0.6	3.3
J17	+++	1.9	0.8	3.4
J18	+++	1.9	0.6	4.2
J19	+++	0.9	0.3	4.0
J20	+++	1.4	0.6	3.3
J21	+++	1.3	0.6	3.2
J22	-	-	-	-
J23	-	-	-	-
J24	-	-	-	-

Eight other isolates cannot dissolve phosphate. The ability of phosphate dissolving can be classified as follows: low dissolution activity (low solubilization) (E<2), moderate dissolution activity (average solubilization) (2<E<3) and high dissolving activity (high solubilization) (E>3) [30]. High P dissolving ability, was noted by J1, J2, J8, J9, J10, J11, J14, J16, J17, J18, J19, J20 and J21 isolates that has a dissolution index P>3. This is consistent with the statement Maryati, 2006 [31] that the higher increase of wide clear zone indicated that bacterial isolates had more superior properties. The ability of bacterial isolates is different in dissolving phosphate, because any phosphate soluble bacteria produce the number and type of different organic acids. Prasetyowati, 2008 [32] stated that an organic acid produced by microorganisms may vary in quality and quantity in dissolving phosphate. The success of phosphate dissolving bacteria depends upon temperature, pH and nutrient supply [33].

In addition to the P elements, essential nitrogen plant nutrients are abundant in the air. However; N cannot be used directly by plants. The ability of bacteria to attach N became one of the alternatives to change the availability of N, so that it can be used directly by plants anchoring pellicle capacity was characterized by the formation of a white ring on NFB media and changes in color of media from the original green color to blue color. The color change in the NFB media was due to the presence of alkaline NH_3, causing the indicator bromothymol blue changes to blue. The process of nitrogen fixation by bacteria will produce ammonia (NH_3). Determination of nitrogen fixation activity was seen from the third dilution. The highest N anchoring capacity of 24

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isolates, was noted on J2 isolates that was equal to 2.8 x 10^3 CFU/mL (Table 5).

Table 5. Nitrogen fixation in the NFB medium by corn-rhizosphere bacterial isolates

Isolates code	Nitrogen fixation (CFU/mL)
N J1	8.1 x 10^2
J2	2.8 x 10^3
J3	6.0 x 10^2
J4	8.2 x 10^2
J5	5.5 x 10^2
J6	-
J7	-
J8	6.0 x 10^2
J9	4.5 x 10^2
J10	9.2 x 10^2
J11	5.5 x 10^2
J12	-
J13	9.2 x 10^2
J14	-
J15	3.7 x 10^2
J16	4.0 x 10^2
J17	-
J18	1.0 x 10^3
J19	1.7 x 10^3
J20	4.0 x 10^2
J21	6.8 x 10^2
J22	1.4 x 10^3
J23	1.0 x 10^3
J24	-

Freire (1984) [34] argues in his research, that the success of the air-N_2 fixation by bacteria depending upon environmental conditions such as pH and temperature. Of the 24 isolates screened, it was obtained 10 isolates that showed high IAA production capacity, namely J17, J8, J15, J9, J16, J24, J13, J19, J3 and J11. Ten isolates were also selected based on their ability to dissolve N and P.

3.3. Testing of selected isolates to corn seed germination

Based on biochemical activity test on the ability to produce IAA, N fixing and P solubilization, it was obtained selected 10 isolates namely J17, J8, J15, J9, J16, J24, J13, J19, J3 and J11, which then tested for corn seed germination. According to Salisbury and Ross, 1995 [35] the crop growth can be measured through the increase of plant height, root length, or by the leaf surface area. The J11, J16 and J19 isolates were not significantly different from the control (water), but the isolates tend to trigger the growth of roots and stems than that of control treatment (Table 6).

The results showed the variance of $p > 0.05$ (0.168). Association of root bacteria to the growing of corn sprouts affect the secretion ability of IAA by plant becomes higher. IAA produced by isolates may affect on root morphology, especially long root causing expansion of nutrient uptake and therefore contributes to plant growth as indicated by the longer plant stem.

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Table 6. Effect of bacterial treatment on root and stem length of corn growth

Isolates Treatment	Growth variable	
	Root length (cm)	Stem length (cm)
J3	10.13 ± 1.44	3.50 ± 1.68
J8	8.63 ± 2.50	4.25 ± 2.02
J9	9.25 ± 2.06	4.25 ± 1.70
J11	11.13 ± 2.72	5.12 ± 0.85
J13	10.50 ± 4.21	4.38 ± 1.49
J15	8.75 ± 0.87	4.88 ± 0.63
J16	11.50 ± 3.11	5.88 ± 1.38
J17	8.13 ± 2.06	4.25 ± 0.96
J19	11.75 ± 1.50	6.00 ± 1.22
J24	8.25 ± 1.32	4.38 ± 1.03
Control (water)	8.00 ± 0.82	3.50 ± 1.29

3.4. Testing inoculation of selected bacteria on the growth of corn plants

The third selected namely J11, J16 and J19 isolates treatment with five replications which was measured for 4 weeks on the growth of corn plants, the following data of plant height and roots length of corn plants can be seen in Table 7.

Tabel.7. Effect of treatments on plant height and length of the roots of corn plants

Treatment	Plant height after 4 weeks (cm)	Roots length (cm)
J11	93.00 ± 8.52	84.60 ± 31.64
J16	95.74 ± 6.08	106.00 ± 12.31
J19	98.60 ± 12.28	122.40 ± 39.83
Control (water)	88.00 ± 5.87	95.60 ± 9.73

J19 treatment, tend to generatee plant height and root length higher than that of other isolates and control (water), even though it was not significantly different by HSD test ($P<0.05$). According to Lakitan, 1996 [36], plant height is a variable of a plant that is often observed both as an indicator of growth or environmental effect or treatment applied. Treatment with J19 isolates had the highest plant height compared with other treatment (Table.7), however; variance test results showed there were no significantly difference among treatments. The results showed the variance of $p>0.05$ (0.285). The variable of root length ofJ19 isolates treatment showed the highest roots length than any other treatment, however; no significantly difference in the diversity among treatments. The results show the variance of $p>0.05$ (0.183). Higher plant roots length is important for plant to support the growth. Previous research revealed that the increase in the volume and roots length of plant is one of the mechanisms to cope with drought stress [37].

3.5. Inoculation testing of selected bacteria on the dry weight of corn plants

Effect of 3 selected isolates treatment on the dry weight of corn plants can be seen in Table 8.

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Table 8. Effect of the treatment of the dry weight of corn plant

Treatment	Dry weight of plant (g)
J11	52.65 ± 13.86
J16	59.18 ± 13.94
J19	63.58 ± 26.57
control (water)	47.90 ± 4.72

The J19 isolates treatment showed the highest dry weight compared with other treatment, but test results showed no significantly difference among treatments (variance of $p>0.05$ (0.480). Calculation of the plant dry weight is important, because the dry weight is used to view the plant metabolism. The plant dry weight may represent the yield, because the leaves and other organs containing metabolites results. Dry weight gain is used as an indicator of plant growth because heavy accumulation of organic compounds synthesized from inorganic compounds that plants water and dried CO_2. Dry weight also overview the plants to perform efficient photosynthesis during the growth process, where 90% of the plant dry weight is the result of photosynthesis [38].

The results showed that the variance did not differ significantly affected by the supply of pure tryptophan. Root exudates may affected the growth and activity of microorganisms in the rhizosphere. The abundant root exudates will encourage the growth of abundant bacteria too. The exudates generated during the 28 days old plant is still in a small amount, so that the supply of tryptophan as IAA precursors also still lower. Lines-Kelly, 2005 [39] states that the concentration of exudates in the rhizosphere environment varies greatly depending upon the age of the plants, In this study the hharvesting time wasalso done at 28 days old plant (late vegetative stage). Belfield and Christine, 2008 [40] stated that the late vegetative stage of corn is the most important phase as it affected the yield significantly if lack of water or nutrient deficiency. As supported by Husen, 2009 [41] also mentioned that the response of corn plants against new biological fertilizer was seen from 35th day old plant to the end of the vegetative period, hence inoculants will provide a real effect on the growth of corn plants after the 35th day.

3.6. Testing inoculation of selected bacteria on the growth of corn plants

16S rRNA PCR amplification product and visualization of 3 selected bacteria can be seenin Table 9.

Table 9. The identification of 3 isolates elected based on BLAST analysis

Isolates Code	Species identity
J11	Uncultured *Bacillus* sp
J16	*Klebsiella oxytoca*
J19	*Pseudomonas nitroreducens*

The three selected bacteria such as J11, J16 and J19 which identified by the BLAST program, showed that the three isolates belonging to species Uncultured *Bacillus* sp J11, whereas J16 and J19 belong to *Klebsiella oxytoca*and *Pseudomonas nitroreducens*, respectively.

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4. Conclusion

In the rhizosphere of corn plants, it was obtained 24 bacterial isolates that showed the ability of IAA producing with the levels ranging from 4.83 to 125.84 ppm. The 16 rhizosphere bacterial isolates capable of dissolving phosphate with phosphate solubility index ranged from 2.1 to 4.6, while 18 rhizosphere bacterial isolates capable of N fixation with the highest N soluble capacity of 2.8 x 10^3 CFU/mL. Application of three selected bacteria such as J11, J16 and J19 as bacterial inoculants were not able to increase the growth of corn plants compared with control treament. Identification three selected bacteria (J11, J16 and J19) were belonging to Uncultured *Bacillus* sp, *Klebsiella oxytoca* and *Pseudomonas nitroreducens*, respectively. Further study observations with different plant maturities (longer than 28 days after planting) is needed to see the effect of bacterial inoculants on plant growth.

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